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Azolla cristata in the Kashmir Himalaya

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ABSTRACT.—The identity of *Azolla* species in the Kashmir Valley has been confusing, with most populations being reported as *Azolla pinnata*. Morphological evidence demonstrates that the species introduced to Kashmir is not *A. pinnata*, but rather *Azolla cristata*, a new species for the Kashmir Valley and a potentially problematic biological invader.

KEY WORDS.—*Azolla caroliniana*, *Azolla cristata*, *Azolla filiculoides*, Kashmir Valley, invasive species

The genus *Azolla* Lam. consists of small, free-floating aquatic ferns, which are invasive in many parts of the world (Garcia-Murillo, 2007), including the Kashmir Valley, where *Azolla* is one of the most prolific invasive alien taxa of aquatic ecosystems (Fig. 1A). The species of *Azolla* that grows in Kashmir has been previously reported as *Azolla pinnata* R. Br. (Mir and Pandit, 2008), referring to the native Indian taxon *A. pinnata* subsp. *asiatica* R.M.K. Saunders & K. Fowler. A detailed study of vegetative and reproductive characters of *Azolla* specimens collected from several water bodies in Kashmir Valley revealed that this species has been incorrectly identified and is, instead, *A. cristata* Kaulf.

MATERIALS AND METHODS

Fresh specimens with sporocarps were collected from five different water bodies of the Kashmir Valley, and deposited in Kashmir University herbarium, KASH (Table 1). Additional material from this herbarium was examined under a stereomicroscope (Zeiss, Discovery V8) and optical trinocular microscope (Leica DMLS2). The branching pattern and leaf trichomes were observed by immersing fresh plants in 95% ethanol for 1 hour. The leaves were then rinsed with water and immersed in concentrated bleaching solution under vacuum for 15 minutes, again rinsed with water, then mounted in lactophenol and observed under the microscope. The structure of glochidia was observed by immersing dry microsporangia in water:ethanol:glycerol (1:1:1) solution for 24 hours. The massulae were then dissociated by gently squashing them under a cover glass. Samples for Scanning Electron Microscopy (SEM) were fixed with 3% glutaraldehyde in 0.2 M phosphate buffer of pH 7.4. The samples

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FIG. 1. A. A population of *Azolla* forming a mat in Dal Lake. B) Sporophyte of *Azolla cristata*. *Reshi & Ahad 2101* (KASH). C) Bicellular leaf-trichome. *Reshi & Ahad 2101* (KASH).

were then fixed in 1% osmium tetraoxide, and graded in acetone series. Fixed samples were dried by critical point method in dry carbon dioxide and examined under the SEM (Hitachi S3000-H).

RESULTS AND DISCUSSION

The plants are free-floating, up to 4 cm long, and polygonal in shape. Sporophytes consist of a thin axial stem, bearing small leaves and delicate brown rootlets up to 6 cm long. Leaves are alternate, imbricate, and bilobed; the hyaline ventral leaf lobe and an aerial chlorophyllous dorsal lobe are covered with short bicellular trichomes and bear an extracellular cavity housing filamentous *Anabaena* (Figs. 1B and 1C). Rootlets are solitary, from stem branching points. Sporocarps are borne in pairs at the base of branches. Microspores are aggregated in stalked massulae, massulae with arrow shaped glochidia (Figs. 2A and 2B). Megasporocarps contain a solitary megaspore; megaspore apparatus with three floats, and granular perine (Fig. 2C).

To understand the identification of these plants that were earlier misidentified as *A. pinnata* in the Kashmir valley (a species of section *Rhizosperma*, characterised by hookless glochidia and megaspore apparatus with nine floats) a short background in *Azolla* taxonomy is necessary. One of the earliest classifications of *Azolla* section *Azolla* by Mettenius (1847) recognized four

TABLE 1. Collection information. Elevations are in meters above sea level. Vouchers are deposited at KASH.

Site	Elevation	Coordinates	Voucher
Anchar Lake	1584	34.23°N, 74.90°E	Reshi & Ahad 2103
Dal Lake	1584	34.60°N, 74.80°E	Ahad & Ganaie 2102
Hokersar wetland	1500	34.05°N, 74.43°E	Reshi & Ahad 2105
Manasbal Lake	1584	34.15°N, 74.41°E	Ahad & Ganaie 2104
Wular Lake	1580	34.20°N, 74.44°E	Reshi & Ahad 2101

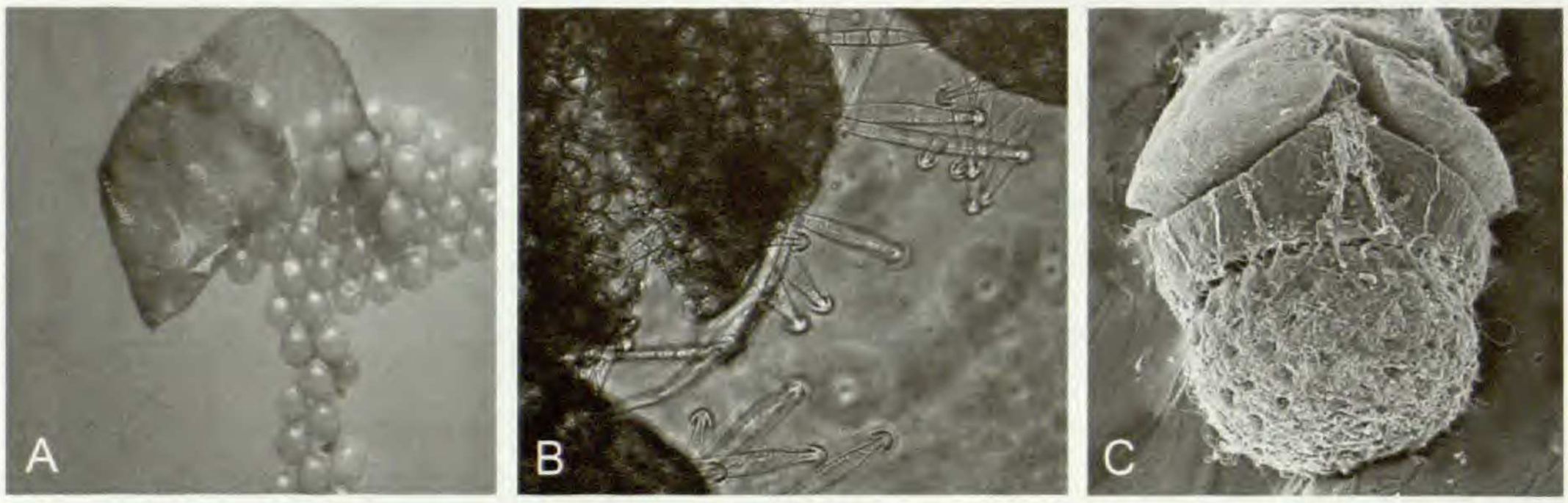

FIG. 2. A. Massulae. *Reshi & Ahad 2101* (KASH). B. Arrow shaped glochidia.*Reshi & Ahad 2101* (KASH). C. SEM of megasporocarp showing perine architecture. *Reshi & Ahad 2101* (KASH).

species: *A. cristata* Kaulf, *A. caroliniana* non Willd., *Azolla magellanica* Willd. (syn. *Azolla filiculoides* Lam.) and *A. microphylla* non Kaulf. Mettenius (1867) reduced the New World species number to two by combining *A. microphylla, A. caroliniana* and *A. cristata* into *A. caroliniana* and at the same time placing *A. magellanica* and *A. rubra* into *A. filiculoides* Lam. Strastburger (1873) upheld the later Mettenius taxonomy and recognised only two species of section *Azolla*: *A. caroliniana* and *A. filiculoides*, which he distinguished on the basis of bicellular trichomes and granular perine present in the former species and unicellular trichomes and warty perine present in the latter. The most widely accepted classification of section *Azolla* is currently that of Svenson (1944), however, who returned to a four-species system: *Azolla caroliniana* Willd., *A. filiculoides* Lam., *A. microphylla* Kaulf. and *A. mexicana* Presl. Recent reassessments of species limits in *Azolla* have found that taxonomic treatment to be unsatisfactory (Dunham and Fowler, 1987; Zimmerman *et al.*, 1989, 1991; Pereira *et al.*, 2001; Evrard and van Hove, 2004). These authors have shown that earlier circumscriptions of these species were based on misapplied names, including the fact that the types of *A. caroliniana* and *A. microphylla* are indistinguishable from the oldest name in the group, *A. filiculoides*. The taxon frequently treated as *A. caroliniana* sensu Mettinius, then, needs a name, and the oldest available is *A. cristata*. Compounding this confusion is the natural variation present in these species, and noticeable environmental plasticity among the populations (Zimmerman *et al.*, 1989; Pereira *et al.*, 2001). Zimmerman *et al.* (1989) demonstrated that in section *Azolla*, *A. filiculoides* was easily distinguishable from the other taxa on the basis of leaf trichome morphology and enzyme electrophoresis pattern. Zimmerman *et al.* (1991) suggested that the taxa frequently treated as *A. microphylla, A. mexicana* and *A. caroliniana* be regarded as a single species, on the basis of RFLPs, isozymes, phosphorus deficiency symptoms and breeding experiment analysis. These results thus agree perfectly with the existence of two species in section *Azolla*, as advocated by Evrard and Van Hove (2004). One is *A. cristata* Kaulf., which includes *A. caroliniana* non Willd., *A. mexicana* Presl and *A. microphylla* non Kaulf.; the other species is *A. filiculoides* Lam. (including *A. rubra* R. Br., often recognized at the rank of variety).

Following this taxonomy, all plants examined were *Azolla cristata,* on the basis of the presence of bicelled trichomes, hook shaped, multiseptate glochidia, and a 3-float megaspore apparatus with a granular perine surface (Figs. 1C, 2B and 2C). It is possible that *A. filiculoides* is also present in the Kashmir Valley (as an introduction), although we know of no documented occurences; populations of *Azolla* section *Azolla* should be carefully examined to determine which species is involved.

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